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Fas and Fas Ligand: A Death Factor and Its Receptor

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I. Introduction

Homeostasis of mammalian tissues is controlled not only by proliferation and differentiation of cells but also by cell death (Raff, 1992). There are two death processes, apoptosis and necrosis (Walker *et al.*, 1988; Wyllie *et al.*, 1980). The death of cells during embryogenesis, metamorphosis, endocrine-dependent tissue atrophy, and normal tissue turnover is called programmed cell death. Most of the programmed cell death which occurs during mammalian development proceeds by apoptosis. Apoptosis can be morphologically and biochemically distinguished from necrosis which occurs during pathological cell death as a result of injury, complement attack, severe hypoxia, hyperthermia, lytic viral infection, and exposure to a variety of toxins. Apoptosis is accompanied by condensation and segmentation of nuclei, loss of plasma membrane microvilli, and extensive degradation of the chromosomal DNA into nucleosome units.

In addition to apoptosis during development (programmed cell death), apoptosis occurs in other systems. For example, in the immune system, the death of thymocytes induced through their antigen–receptor complex or by glucocorticoid occurs by an apoptotic process (Golstein *et al.*, 1991). Tumor regression by the immune system is also mediated by apoptosis; that is, cytotoxic T lymphocytes (CTL) or natural killer cells (NK) as well as tumor necrosis factor (TNF) or lymphotoxin (LT) induce apoptosis in the target cells. Furthermore, low doses of UV or γ-ray irradiation or antitumor chemical drugs cause apoptosis of tumor cells (Hickman, 1992; Wyllie *et al.*, 1980).

Programmed cell death has been extensively studied in the live nematode, *Caenorhabditis elegans* (Ellis *et al.*, 1991), in which the division and death of cells can be followed under the microscope. Many mutants of the death process have been identified, and their molecular analyses have indicated that many gene products are involved in various aspects of cell death in *C. elegans*. On the other hand, the molecular mechanism of cell death in the mammalian system is poorly understood despite its importance during development.

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The Fas antigen is expressed on the surface of various mammalian

cells. It is a member of the TNF/nerve growth factor (NGF) receptor family and transduces the apoptotic signal (Itoh *et al.*, 1991; Watanabe-Fukunaga *et al.*, 1992b). Molecular analysis of the Fas gene has indicated that it is the structural gene for the mouse *lpr* (lymphoproliferation) mutation (Adachi *et al.*, 1993; Watanabe-Fukunaga *et al.*, 1992a). We have identified a natural Fas ligand in a CTL cell line and showed that it is a member of the TNF family (Suda *et al.*, 1993). Here, the Fas/Fas ligand system is summarized and its physiological role is discussed.

II. Fas Antigen

The Fas antigen (Fas) (Yonehara *et al.*, 1989), also called APO-1 antigen (Trauth *et al.*, 1989), is a cell-surface protein belonging to the TNF/NGF receptor family (Itoh *et al.*, 1991; Oehm *et al.*, 1992; Nagata, 1993). As shown in Fig. 1, the members of this family include two TNF receptors (types I or 55K and type II or 75K, respectively) (Schall *et al.*, 1990; Smith *et al.*, 1990); the low-affinity NGF receptor (Johnson *et al.*, 1986); the B-cell antigen CD40 (Stamenkovic *et al.*, 1989); the T-cell antigen OX40 (Mallett *et al.*, 1990), CD27 (Camerini *et al.*, 1991), 4-1BB (Kwon and Weissman, 1989); and the Hodgkin's lymphoma cell-surface antigen CD30 (Dürkop *et al.*, 1992). The extracellular regions of members in this family are rich in cysteine residues, and they can be divided into three to six subdomains. The amino acid sequence of the extracellular region is relatively conserved (about 24–30% homology), whereas the cytoplasmic region is not, except for some similarity between Fas and the TNF type I receptor (Itoh *et al.*, 1991). The TNF and NGF receptors were identified as cytokine receptors. Fas, CD40, CD27, and CD30 are proteins which are recognized by specific monoclonal antibodies. Molecular cloning of the ligands for CD40, CD27, CD30, and 4-1BB (Armitage *et al.*, 1992; Goodwin *et al.*, 1993a,b; Smith *et al.* 1993) indicated that they are TNF-related type II membrane proteins and constitute a novel cytokine family (Farrah and Smith, 1992). As described below, the Fas ligand also turns out to be a member of the TNF family.

III. Expression of Fas

Activated human T and B cells abundantly express Fas (Trauth *et al.*, 1989). Lymphoblastoid cells transformed with human T-cell leukemia virus (HTLV)-I (Debatin *et al.*, 1990), human immunodeficiency virus (HIV) (Kobayashi *et al.*, 1990), or Epstein-Barr virus (EBV) (Falk *et al.*, 1992) highly express Fas. Some other tumor cell lines

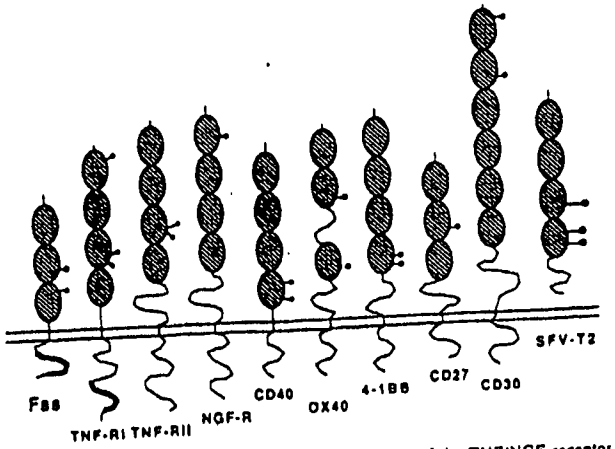

FIG. 1. The Fas/TNF/NGF receptor family. Members of the TNF/NGF receptor family are schematically shown. These include Fas; TNF type I and type II receptors; low-affinity NGF receptor; B-cell antigen CD40; T-cell antigens OX40, 4-1BB, and CD27; Hodgkin's lymphoma antigen CD30; and the soluble protein coded by Shope fibroma virus (SFV-T2). The shaded regions represent cysteine-rich subdomains, of which each member of the family contains three to six. A domain of about 80 amino acids in the cytoplasmic regions of Fas and the type I TNF receptor has some similarity, and it is shown as a bold line. ♆ indicates N-glycosylation sites.

such as human myeloid leukemia U937 (Yonehara *et al.*, 1989), human squamous carcinoma CHU-2 (Itoh *et al.*, 1991), and SV40-transformed mouse macrophage BAM3 cells (Watanabe-Fukunaga *et al.*, 1992b) express Fas, although the expression level is low compared with that of the lymphoblastoid cell lines. The expression of Fas is upregulated by interferon-γ (IFN-γ) in the mouse macrophage BAM3, human adenocarcinoma HT-29 and mouse fibroblast L929 cell lines (Itoh *et al.*, 1991; Watanabe-Fukunaga *et al.*, 1992b), or by a combination of IFN-γ and TNF-α in human tonsillar B cells (Möller *et al.*, 1993).

The tissue distribution of the Fas mRNA in the mouse has been examined (Watanabe-Fukunaga *et al.*, 1992b). The Fas mRNA was detected abundantly in the thymus, heart, liver, and ovary of 8-week-old adult mice, but not in the brain, bone marrow, and spleen. In thymocytes, Fas is expressed in almost all populations except for double-negative ($CD4^-$ $CD8^-$) thymocytes (Drappa *et al.*, 1993; Oga-

sawara *et al.*, 1993; J. Ogasawara, T. Suda, and S. Nagata, unpublished results).

IV. Mutation of the Fas Gene in *lpr* Mice

Southern hybridization of genomic DNA indicated that there is only one chromosomal gene for Fas in human and mouse chromosomes (Adachi *et al.*, 1993). *In situ* hybridization localized the human gene on chromosome 10q24.1 (Inazawa *et al.*, 1992), and interspecific backcross analysis indicated that the mouse Fas gene is in the region of chromosome 19, which is homologous to human 10q24.1 (Watanabe-Fukunaga *et al.*, 1992b). Referring the location of the mouse Fas gene to the mouse genomic database (GBASE), it was found that the Fas gene is close to the *lpr* locus (Watanabe *et al.*, 1991). There are two known allelic mutations, *lpr* and lpr^{cg}, at the *lpr* locus. These mutants have a similar phenotype, but lpr^{cg} slightly complements the *gld* mutation in double-heterozygotes between *lpr* and *gld* mutations (Matsuzawa *et al.*, 1990). Northern hybridization of the thymus and liver from *lpr* mice showed little expression of the Fas mRNA (Watanabe-Fukunaga *et al.*, 1992a). Accordingly, flow cytometry using anti-mouse Fas antibody hardly detected the Fas protein on the thymocytes from *lpr* mice (Drappa *et al.*, 1993; Ogasawara *et al.*, 1993). Since Southern hybridization of the chromosomal DNA suggested a distinct rearrangement of the Fas gene in *lpr* mice, the chromosomal gene was molecularly cloned from the wild-type and *lpr* mice (Adachi *et al.*, 1993). The mouse Fas gene consists of over 70 kb and is split by 9 exons (R. Watanabe-Fukunaga and S. Nagata, unpublished results). Restriction enzyme mapping of the Fas gene from *lpr* mice indicated that the promoter and exons of the Fas gene in this mouse are intact. However, an early transposable element (ETn) of 5.4 kb was inserted in intron 2 of the Fas gene. The ETn is a mouse endogenous retrovirus, of which about 1000 copies can be found in the mouse genome (Brulet *et al.*, 1983). Although the ETn does not carry a meaningful open reading frame, it has long terminal repeat (LTR) sequences (about 300 bp) at both the 5′ and 3′ termini. This LTR sequence contains a poly(A) adenylation signal (AATAAA) which terminates the transcription at this region. In fact, short mRNA's of about 1.0 kb coding for exons 1 and 2 of the Fas gene were abundant in the thymus and liver of the *lpr* mice (Adachi *et al.*, 1993). Furthermore, inserting the ETn into an intron of a mammalian expression vector dramatically, but not completely, reduced the expression efficiency in mammalian cells. These results indicate that, in *lpr* mice, an insertion of an ETn into an intron of the Fas gene greatly reduces the expression of the functional Fas mRNA, but its mutation is leaky. Later, several other groups reached the same conclusion by analyzing the Fas transcript in *lpr* mice by means of the reverse polymerase chain reaction (Chu *et al.*, 1993; Kobayashi *et al.*, 1993; Wu *et al.*, 1993).

In contrast to the *lpr* mice, lpr^{cg} mice express the Fas mRNA of normal size as abundantly as the wild type (Watanabe-Fukunaga *et al.*, 1992a). However, this mRNA carries a point mutation of T to A, which causes the replacement of isoleucine with asparagine in the Fas cytoplasmic region. This mutation is in the domain which has similarity with the TNF type I receptor (see below), and it abolishes the ability of Fas to transduce the apoptotic signal (Watanabe-Fukunaga *et al.*, 1992a). Furthermore, when the corresponding amino acid (valine-238) of the human Fas was mutated to asparagine, it could not transduce the apoptotic signal into cells (Itoh and Nagata, 1993).

V. Fas-Mediated Apoptosis *in Vitro* and *in Vivo*

To assess the function of Fas, mouse cell transformants constitutively expressing human Fas were established using various mouse cell lines as host cells (Itoh *et al.*, 1991). When the transformed cells were treated with anti-human Fas antibody, cells expressing human Fas, but not the parental mouse cells, died within 5 hr. Examination of the dying cells under an electron microscope revealed extensive condensation and fragmentation of the nuclei, which is characteristic of apoptosis. The chromosomal DNA started to degrade in a laddered fashion after a 2-hr incubation with the anti-Fas antibody. A human Fas expression plasmid has also been introduced into a mouse interleukin-3(IL-3)-dependent myeloid leukemia FDC-P1 cell line (Itoh *et al.*, 1993). Although the transformed cells died due to IL-3 depletion, they did so over 36 hr, as observed with the parental FDC-P1 cells. On the other hand, exposure to the anti-human Fas antibody killed the cells within 5 hr in the presence of IL-3. From these results, we concluded that Fas actively mediates the apoptotic signal into cells, and the anti-Fas antibody works as agonist.

The anti-Fas antibody has lethal activity *in vivo* (Ogasawara *et al.*, 1993). We established several hamster monoclonal antibodies against mouse Fas. One of them had cytolytic activity *in vitro*. When this antibody is intraperitoneally injected into mice, the wild-type mice but neither *lpr* nor lpr^{cg} mice died within 5–6 hr. These results clearly indicate that the lethal effect of the anti-Fas antibody is due to binding of the antibody to Fas to activate the death pathway and not due to a substance(s) such as endotoxin contaminated with the antibody. Furthermore, the fact that lpr^{cg} mice expressing the nonfunctional Fas are

resistant to the lethal effect of the antibody suggests little involvement of the complement system in this killing process. Biochemical analysis of sera from the dying mice showed a specific and dramatic increases of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels shortly after injection of the antibody, suggesting liver injury. Accordingly, histological and electron microscope analyses of the tissues indicated that hepatocytes were killed by apoptosis (Fig. 2). The effect of the anti-Fas antibody *in vivo* seems to be a direct effect on the liver because the anti-Fas antibody also caused apoptosis in primary cultures of hepatocytes (R. Ni, Y. Tomita, A. Ichihara, K. Ishimura, J. Ogasawara, and S. Nagata, unpublished results). These results indicate that the Fas expressed in mouse tissues (at least in the liver) is competent in transducing the apoptotic signal into cells.

VI. Signal Transduction Mediated by Fas

The apoptotic signal is induced by the binding of anti-Fas or anti-APO-1 antibody, or the Fas ligand, to Fas. The anti-human Fas antibody is an IgM class antibody which is an immunoglobulin pentamer, whereas the anti-APO-1 antibody is an IgG_3 class antibody which tends to aggregate. The $F(ab')_2$ fragment or other isotypes of the anti-APO-1 antibody hardly induce apoptosis of cells expressing Fas (Dhein *et al.*, 1992). On the other hand, the cytotoxic activity of the inactive anti-APO-1 antibody was reconstituted by cross-linking the antigen with a second antibody or with protein A. These results indicate that Fas dimerization alone is not sufficient to transduce the apoptotic signal. It seems that the oligomerization of at least three Fas molecules is a biologically relevant complex in generating an intracellular signal. As described below, the fact that Fas ligand is a TNF-related molecule which exists as a trimer (Smith and Baglioni, 1987) agrees with this hypothesis.

The cytoplasmic domain of Fas consists of 145 amino acids, in which no motif for enzymatic activity such as kinases or phosphatase can be found. However, about 70 amino acids in this region have significant similarity with a part of the cytoplasmic region of the type I, but not the type II, TNF receptor (Itoh *et al.*, 1991). TNF has numerous biological functions, including cytotoxic and proliferative activities (Old, 1985). Tartaglia *et al.* (1991) have shown that the type I receptor is mainly responsible for the cytotoxic activity of TNF, while the type II receptor mediates the proliferation signal in thymocytes. The similarity of Fas and the type I TNF receptor in their cytoplasmic regions therefore suggests an important role of this domain for apop-

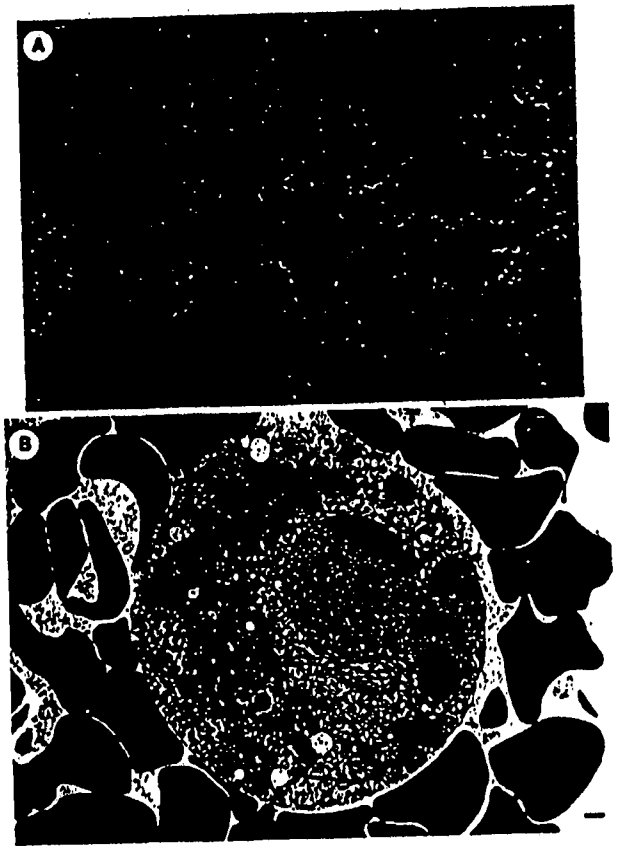

FIG. 2. The Fas-mediated apoptosis of hepatocytes in vivo. The purified anti-mouse Fas antibody (100 μg) was subcutaneously injected into mice. At 2 hr after injection, the liver section was stained with hematoxylin and eosin (A), which shows focal hemorrhage and necrosis. Only a few normal hepatocytes remained, and most hepatocytes carry pyknotic nuclei. (B) A liver section examined under a transmission electron microscope. The affected hepatocytes show the condensed and fragmented nuclei characteristic of apoptosis.

totic signal transduction. In fact, analyses of serial deletions and point mutations in the Fas protein have indicated that the domain conserved between Fas and the type I TNF receptor is essential for the function of Fas (Itoh and Nagata, 1993). Observations of the human type I TNF receptor have indicated that the domain homologous to Fas is responsible and sufficient for TNF-induced cytolytic activity (Tartaglia *et al.*, 1993), which agrees with our conclusion. Furthermore, the mutational analysis of Fas revealed an inhibitory domain for apoptosis in the C-terminus. That is, a Fas mutant lacking 15 amino acids from the C-terminus was an upmutant, in which about 10 times less anti-Fas antibody than that required for the wild-type Fas was sufficient to induce apoptosis (Itoh and Nagata, 1993). It is possible that association of accessory molecule(s) or modification of Fas at this region down-regulates the activity of Fas to transduce the apoptotic signal.

VII. Fas Ligand

As described above, the structure of Fas suggested that it is a receptor for an unknown cytokine. Rouvier *et al.* (1993) have established a CTL hybridoma cell line (PC60-d10S, abbreviated d10S) which has cytotoxic activity against thymocytes from wild-type, but not *lpr* mice, suggesting the presence of Fas ligand on its surface. To confirm the expression of Fas ligand in this cell line, we prepared a soluble form (Fas-Fc) of Fas by fusing the extracellular region of Fas to the Fc region of human IgG. The fusion protein inhibited the Fas-dependent CTL activity of d10S cells in a dose-dependent manner, and the Fas ligand was detected by FACS on the cell surface of d10S cells using labeled Fas-Fc (Suda *et al.*, 1993). The Fas ligand was then purified to homogeneity by affinity chromatography using Fas-Fc, and we showed that the purified protein had cytolytic activity against cells expressing Fas (Suda and Nagata, 1994). We then isolated the Fas ligand cDNA from the d10S cell line using the panning procedure (Suda *et al.*, 1993). The recombinant Fas ligand expressed in COS cells induced apoptosis of cells expressing Fas. The amino acid sequence deduced from the nucleotide sequence of the cDNA indicated that the Fas ligand is a TNF-related type II membrane protein (Suda *et al.*, 1993).

As shown in Fig. 3, members of the TNF family include Fas ligand, TNF, LT, and ligands for CD40, CD30, and CD27. TNF was originally identified as a soluble cytokine (Pennica *et al.*, 1984), which works as a trimer (Smith and Baglioni, 1987). However, it was later shown that TNF is synthesized as a type II membrane protein which can be cleaved to produce a soluble form (Kriegler *et al.*, 1988). LT consists of LTα and LTβ and is expressed in certain CTL (Androlewicz *et al.*,

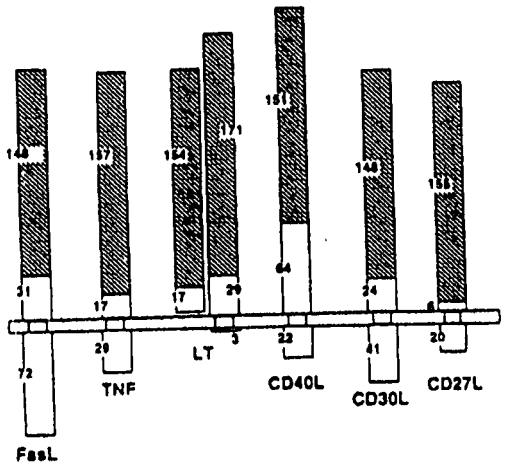

FIG. 3. The TNF family. The members of the TNF family are schematically shown. The members include the Fas ligand (FasL), membrane-bound TNF, lymphotoxin (LT) which consists of LTα and LTβ, CD40 ligand (CD40L), CD30 ligand (CD30L), and CD27 ligand (CD27L). The shaded regions have significant similarity. The numbers indicate the amino acid number of the conserved, the spacer, and intracellular regions.

1992). LTα, also called TNF-β, is produced as a soluble cytokine with a signal sequence (Gray *et al.*, 1984), while LTβ is a type II membrane protein (Browning *et al.*, 1993). LTα and LTβ associate on the cell surface probably as a trimer (Androlewicz *et al.*, 1992) and bind to a newly identified receptor which belongs to the TNF/NGF receptor family (Crowe *et al.*, 1994). The ligands for CD40, CD30, CD27, and 4-1BB are type II membrane proteins expressed in activated T cells (Armitage *et al.*, 1992; Goodwin *et al.*, 1993a,b; Smith *et al.*, 1993). When the Fas ligand is overproduced in COS cells, the soluble form of the Fas ligand which can actively induce apoptosis can be found in supernatant (Suda *et al.*, 1993; Suda and Nagata, 1994). These results suggest that under abnormal conditions, the soluble form of the Fas ligand can be produced in the body as found in the TNF system (Old, 1985).

The tertiary structure of TNF has been extensively studied. It forms an elongated, antiparallel β-pleated sheet sandwich with a jellyroll topology (Banner *et al.*, 1993; Eck and Sprang, 1989; Eck *et al.*, 1992). The significant conservation of the amino acid sequence among members suggests that others of the family, including the Fas ligand, have a structure similar to that TNF and work as a trimer. However, despite the high similarity between Fas ligand and TNF (about 30% identical at the amino acid sequence level), Fas ligand does not bind to the TNF receptor (Suda *et al.*, 1993).

VIII. Physiological Roles of the Fas System

Since Fas was identified as a cell-surface protein which mediates apoptosis (Itoh *et al.*, 1991), considerable progress has been made regarding its physiological role. Our finding that the Fas gene is the structural gene for the *lpr* mutation pointed to the important role of Fas in the development of T cells. However, it remains controversial at which step of T-cell development Fas is involved. Immature T cells are killed by apoptosis in at least two steps during development in the thymus (Ramsdell and Fowlkers, 1990). Those T cells carrying T-cell receptors which do not recognize self-MHC antigens as a restriction element are killed or "neglected," while the T cells recognizing the self antigens are killed by a process called "negative selection." Analysis of thymic T-cell development in wild-type and *lpr* mice has suggested that the neglected thymocytes escape from apoptosis in the thymus of *lpr* mice and then migrate to the periphery (Zhou *et al.*, 1993). On the other hand, Herron *et al.* (1993) reported that the development of T cell in the thymus is relatively normal in *lpr* mice. These different observations may be partly due to the leakiness of the *lpr* mutation as mentioned above. In addition to being expressed in thymocytes, Fas is expressed in activated mature T cells (Trauth *et al.*, 1989), and the prolonged activation of T cells leads the cells susceptible to cytolytic activity of anti-Fas antibody (Klas *et al.*, 1993; Owen-Schaub *et al.*, 1992). Since mature T cells from *lpr* mice are resistant to anti-CD3-stimulated suicide, Russell *et al.* (1993) suggested a role of Fas-mediated apoptosis in the induction of peripheral tolerance and/or in the antigen-stimulated suicide of mature T cells.

Fas is expressed in other tissues such as the liver, heart, and lung (Watanabe *et al.*, 1991). Although these organs are rather stable, and no apparent abnormal phenotypes are seen in these tissues of *lpr* mice, Fas may also be involved in development and/or turnover in these tissues. Since abnormal activation of Fas causes severe tissue damage (Ogasawara *et al.*, 1993), it is possible that the Fas system is involved in many human autoimmune diseases such as fulminant hepatitis. In this regard, it is notable that a particular CTL cell line induces apoptosis in hepatocytes and causes fulminant hepatitis (Ando *et al.*, 1993; Chisari, 1992). If involvement of the Fas system in human dis-

eases is proven, antagonistic antibodies against Fas or Fas ligand, or the soluble form of Fas, could be used in a clinical setting.

The Fas ligand is expressed in some CTL cell lines and in activated splenocytes (Suda *et al.*, 1993), suggesting an important role of the Fas system in CTL-mediated cytotoxicity. Two mechanisms for CTL-mediated cytotoxicity are known (Apasov *et al.*, 1993; Golstein *et al.*, 1991; Podack *et al.*, 1991). One is a Ca^{2+}-dependent pathway in which perforin plays an important role. The other pathway is a Ca^{2+}-independent pathway. In the perforin-knocked out mice, the spleen cells still showed some Ca^{2+}-independent CTL activity (Kägi *et al.*, 1994a). Fas ligand can kill the cells independently of Ca^{2+} and Kägi *et al.* (1994b) recently showed that the residual CTL activity remaining in the perforin-knocked out mice is due to the Fas ligand expressed in CTL.

Mice carrying the *gld* mutation show phenotypes similar to *lpr* (Cohen and Eisenberg, 1991). Allen *et al.* (1990) suggested that *gld* and *lpr* are mutations of an interacting pair of molecules. As shown above, the Fas gene is the structural gene for *lpr*, and Fas is the receptor for Fas ligand. Recently, we have found that *gld* mice carry a mutation in the Fas ligand gene (Takahashi *et al.*, 1994). The mutations in Fas (*lpr* mutation) or Fas ligand (*gld* mutation) causes lymphadenopathy and autoimmune disease, and the Fas ligand was found in CTL. These results imply that the Fas/Fas ligand system involved in the T-cell development plays an important role in CTL-mediated cytotoxicity. It is possible that the killing process of autoreactive T cells in T-cell development and the killing process of tumor cells by CTL may proceed by a similar mechanism. As shown schematically in Fig. 4, self, tumor, or virus antigens in the target cells may activate effector cells (CTL) through the T-cell receptor to induce the expression of the Fas ligand gene. Fas ligand then binds to Fas on the target cells, causing apoptosis. In addition to lymphocytes, Fas and the Fas ligand are expressed in other tissues such as the liver, lung, and heart, suggesting that a similar mechanism operates to remove unnecessary or toxic cells from these tissues during development.

IX. Perspectives

We demonstrated that Fas ligand is a death factor, and Fas is its receptor. These results indicate that just as growth factor and its receptor regulate cell proliferation, cell death or apoptosis is regulated by a death factor and its receptor (Fig. 5). The growth and differentiation of cells are controlled by signals such as activation of kinases, Ca^{2+} mobilization, or cAMP formation, which are stimulated by growth

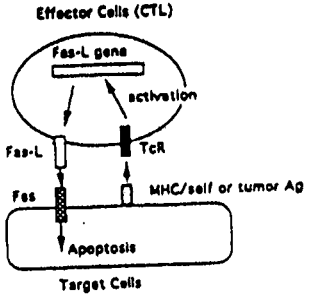

FIG. 4. A model for the Fas-mediated cytotoxicity of CTL. A proposed mechanism for the Fas-mediated cytotoxicity in the CTL system is schematically shown. The target cells express the self, tumor, or virus antigen as a complex with MHC, which interacts with the T-cell receptor (TCR) on CTL. This interaction activates the CTL and induces the expression of the Fas ligand (Fas-L) gene. The Fas-L expressed on the cell surface of the CTL then binds to Fas on the target cells and induces its apoptosis.

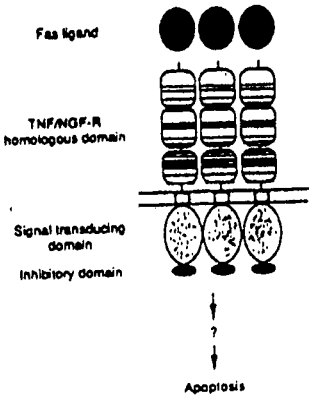

FIG. 5. Fas-mediated apoptosis. Fas and the Fas ligand are schematically shown. The Fas ligand binds to Fas on the cell surface probably as a trimer and activates apoptotic signal transduction. In the cytoplasmic region of Fas, a region of about 80 amino acids is responsible for the signal transduction, while the C-terminal domain (about 15 amino acids) inhibits apoptosis.

and differentiation factors. Currently, the kinds of signaling molecules involved in Fas-mediated apoptosis are unknown. Fas may activate a similar signal transducer, or utilize a completely different set of molecules. Since overexpression of the bcl-2 oncogene product partially inhibits Fas-mediated apoptosis (Itoh *et al.*, 1993), bcl-2 should interact somewhere in the signal-transducing pathway activated by the Fas system. Elucidation of the apoptotic signal transduction mechanism mediated by Fas may reveal a novel mechanism.

The gain-of-function mutation of the growth factor system causes cellular transformation, whereas the loss-of-function mutation of the Fas system (*lpr* mutation) causes lymphadenopathy. In this regard, Fas and the Fas ligand may be considered as tumor suppressor genes. The loss-of-function mutation in the growth factor system causes the disappearance or dysfunction of specific cells. As pointed out above, it is possible that abnormal activation (gain of function mutation) of the Fas or Fas ligand causes fulminant hepatitis or other diseases such as CTL-mediated autoimmune diseases.

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ADVANCES IN IMMUNOLOGY, VOL. 57

Interleukin-5 and Its Receptor System: Implications in the Immune System and Inflammation

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I. Introduction

The immune system to infectious microbes is regulated by a series of interactions among T cells, B cells, and macrophages. During this process, B cells proliferate and differentiate into plasma cells which produce antibodies against distinct antigenic determinants of the antigen, and the antibodies produced play a key role in the humoral immune response against invading microorganisms. The B-cell response to an antigen is regulated by a helper T cell responding to, and specific for, the same antigen molecule. Helper T cells recognize antigenic peptide in the context of class II major histocompatibility complex (MHC) molecules on accessory cells and/or B cells (Brown *et al.*, 1993) and secrete several soluble factors including interleukin-4 (IL-4), IL-5, and IL-6 which can induce B-cell growth and maturation of B cells (reviewed by Howard and Paul, 1983; Melchers and Anderson, 1986; Kishimoto and Hirano, 1988; Takatsu, 1988; Paul and Ohara, 1987; Vitetta *et al.*, 1984).

Mouse interleukin-5 (mIL-5) is a glycoprotein induced in T cells after stimulation with an antigen, such as *Mycobacterium tuberculosis* (Tominaga *et al.*, 1988) or *Toxocara canis* (Y. Yamaguchi *et al.*, 1990a), and in mast cells upon stimulation with allergen/IgE complex or calcium ionophores (Plaut *et al.*, 1989). The study of mIL-5 originated from the search for the B-cell differentiation factor that induces antigen-primed B cells to differentiate into antigen-specific antibody-producing cells or proliferation of BCL, B-cell tumor cells (Takatsu *et al.*, 1980a; reviewed by Takatsu *et al.*, 1988). This molecule was identified as a cytokine that has pleiotropic activities on various target cells including B cells, T cells, eosinophils, and basophils by the use of recombinant IL-5 and monoclonal antibody (mAb) to IL-5 (Harada *et al.*, 1987a; Schumacher *et al.*, 1988). Two mAbs, NC17 and TRF4, have been widely used because of their ability to neutralize mIL-5 function both *in vitro* and *in vivo* and hIL-5 function *in vitro* (Abrams *et al.*, 1992; Coffman *et al.*, 1989a; Hitoshi *et al.*, 1991; Mita *et al.*, 1989b). A number of mIL-5-dependent mouse B-cell lines have

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